HHS Public Access

Author manuscript

Semin Oncol. Author manuscript; available in PMC 2016 August 01.

Published in final edited form as:

Semin Oncol. 2015 August ; 42(4): 562–572. doi:10.1053/j.seminoncol.2015.05.012.

Enhancing Cancer Immunotherapy Via Activation of Innate Immunity

Jacob L. Goldberg, BS[1] and **Paul M. Sondel, MD, PhD**[1,2,3]

[1]Department of Pediatrics, The University of Wisconsin, Madison WI

[2]Department of Human Oncology, The University of Wisconsin, Madison WI

[3]Department of Genetics, The University of Wisconsin, Madison WI

Abstract

Given recent technological advances and advances in our understanding of cancer, immunotherapy of cancer is being used with clear clinical benefit. The immunosuppression accompanying cancer itself, as well as with current cancer treatment with radiation or chemotherapy, impairs adaptive immune effectors to a greater extent than innate effector cells. In addition to being less suppressed, innate immune cells are capable of being enhanced via immune-stimulatory regimens. Most strategies being investigated to promote innate immune responses against cancer do not require complex, patient-specific, ex-vivo cellular or molecular creation of therapeutic agents; thus they can, generally, be used as “off the shelf” therapeutics that could be administered by most cancer clinics. Successful applications of innate immunotherapy in the clinic have effectively targeted components of the innate immune response. Preclinical data demonstrate how initiation of innate immune responses can lead to subsequent adaptive long-term cancer immunity. We hypothesize that integration of innate immune activation strategies into combination therapies for cancer treatment will lead to more effective and long term clinical benefit.

Introduction/Background: Rationale for targeting innate immunity for enhancement of cancer immunotherapy

Except for the example of “Graft-versus-Leukemia” (GVL) following allogeneic bone marrow transplant[1], prior to ~1985 few preclinical advances in cancer immunotherapy were being translated clinically. This was due to limitations in our: 1) understanding of cancer; 2) animal models, which were not simulating clinical cancer treatment; and 3) technologic ability to create agents in sufficient quantity to impact cancer. In this regard, the ability to

Corresponding Author: Paul M. Sondel MD PhD, 4159 Wisconsin Institute for Medical Research, and UW Carbone Cancer Center, University of Wisconsin Madison, 1111 Highland Ave. Madison WI, 53705, Ph: 608-263-9069, FAX: 608-263-4226, pmsondel@humonc.wisc.edu.

There are no conflicts of interests declared for either author.

make recombinant human cytokines [interferon (IFNs), interleukin-2 (IL2)] and monoclonal antibodies (mAbs) advanced this research greatly. Thus clinical immunotherapy is being more frequently used with clinical benefit[2].

Several barriers to efficacy remain however. First, when cancer is diagnosed, the growing neoplasm has already escaped from the immune system, and thus has been selected for its ability to not be recognized or destroyed by endogenous immunity[3]. Thus, in a subset of patients, effective immunotherapy requires the induction of mechanisms far more potent than those that the ineffective endogenous immune response tried to muster. The 2nd barrier is that cancer itself, and current cancer treatments, are highly immunosuppressive, particularly to the adaptive (T-cell) response[4]. Although current data with immune checkpoint inhibitors demonstrate potent restoration of T cell anti-tumor immunity in subsets of advanced cancer patients, nevertheless, innate immune cells [especially natural killer (NK) cells and macrophages], are much less suppressed[5] and are thus attractive effectors as part of an immunotherapeutic strategy. The 3rd barrier is that some of the most potent current cellular therapy approaches require local, patient-specific, high-tech, good-manufacturing practice (GMP)-lab support that is not available for patients treated at most cancer clinics. In order to potentially enable broader application of immunotherapy, there are advantages in strategies that combine reagents that could be stored in any hospital/clinic pharmacy, and be readily applied “off the shelf” for patients worldwide.

A number of immunotherapeutic approaches towards enhancing innate immunity have demonstrated clinical efficacy. In this review, we describe strategies in which innate immune cells have been successfully augmented as part of an immunotherapy regimen. In addition to stimulation of adaptive immunity, we hypothesize that many forms of clinically successful immunotherapy of cancer will likely involve components of innate immune stimulation.

Components and biology of innate immunity

Natural Killer (NK) Cell Biology

At the interface of a developing cancer and its interaction with the immune system, NK cells play a central role in cancer elimination[6]. In mouse models, the role of the NK cell in preventing metastatic dissemination has been clarified[7]. A role for NK cells in the prevention of spontaneous cancer is supported by their cell surface expression of Natural Killer Group 2, Member D (NKG2D) a receptor capable of recognizing signs of stressed/pre-malignant cells. NK cells, unlike the B and T cells of adaptive immunity, are capable of spontaneously destroying cancerous cells without prior sensitization. This unique capability is the result of the mechanisms by which NK cells target cancerous cells for destruction.

The cytolytic activity of NK cells, mediated in part by pre-synthesized granules, requires a balance between “activating” signals and the lack of “inhibitory” signals. The main inhibitory signals in humans, which function to prevent the destruction of “self” tissues, are the inhibitory killer cell immunoglobulin-like receptors (KIRs). The classical ligands for the NK cell’s inhibitory KIR are certain major histocompatibility complex (MHC) class I surface molecules. These MHC-class I molecules are polymorphic cell surface molecules

found on all mature nucleated cells. In contrast to mature $CD4^+$ or $CD8^+$ T cells, which are activated upon presentation with MHC class II or class I bound antigen, NK cells are inhibited from killing when their inhibitory KIR specifically recognize their cognate specific MHC class I molecules. By circumventing this inhibitory mechanism, "naïve" NK cells can activate and lyse tumor cells that lack the MHC class I "off switch." These receptor interactions also explain the wide variability in NK cell activity in the population, as an individual's NK KIR repertoire and MHC I ligands, are encoded on separate chromosomes and independently inherited. Thus different degrees of activating or inhibitory interactions are determined by an individual's KIR repertoire as well as the spectrum of MHC class I molecules the NK cells encounter. These important relationships were first identified clinically in the setting of the allogeneic graft-vs.-leukemia effect for recipients of T-cell depleted allogeneic bone marrow transplantation (BMT).

Neutrophils

Neutrophils are short-lived granulocytes well recognized for their role in the immediate response to a bacterial or fungal inoculation. Although neutrophil activity may potentially be incorporated into effective immunotherapies, their functions can at times promote tumors. In the tumor microenvironment, up-regulation of neutrophil chemotactic factors augments the presence of these short-lived cells. The presence of neutrophils within the tumor microenvironment has been significantly associated with worse prognosis in a number of solid tumors[8, 9]. Neutrophils have been implicated in tumor initiation, via the genotoxic effects of neutrophil generated reactive oxygen species. Neutrophils can also foster tumor progression. For example, some tumor cells secrete IL-8, recruiting neutrophils to the tumor site[10]. The pro-tumoral role of neutrophils includes: inflammatory cell recruitment, tumor cell proliferation, tumor angiogenesis, enhanced invasiveness, and neutrophil-aided extravasation leading to metastasis[11]. Neutrophil elastase – a major anti-bacterial effector mechanism of the cell – can lead to tumor cell proliferation if internalized by the tumor cell[12]. Since they have effector function and also have Fc receptors, neutrophils can mediate Antibody Dependent Cell-mediated Cytotoxicity (ADCC). Some have suggested that most of the anti-tumor effect of human neutrophils, demonstrated via ADCC, is primarily an *in vitro* finding and may not play a substantial role *in vivo*[13]. However, mouse work indicates that tumor associated neutrophils may differentially polarize towards an anti-tumor function, capable of tumor cytotoxicity[14]. Furthermore, clinical data from Cheung and colleagues have demonstrated in sequential trials that the addition of GM-CSF to tumor-reactive mAb seems to provide augmented antitumor efficacy[15].

Macrophages

As observed for neutrophils, macrophage functions may potentially be incorporated into cancer immunotherapies, but their native activities are often tumor promoting. Most tumors (human and mouse) are heavily infiltrated with immunosuppressive M2 macrophages that contribute to tumor progression[16], with phenotype and function distinct from activated effector M1 macrophages that can destroy tumors. Macrophages follow two different patterns of activation: M1 macrophages are effector cells involved in protection from infection and have a tumor-destroying role; M2 macrophages are involved in wound healing and thus have a pro-tumor role. The pro-tumor role of the M2 macrophages involves

immunosuppression (preventing T-cell cytotoxicity), and promoting tumor growth via modulation of the tumor microenvironment. M2 macrophages have also been implicated in promotion of metastatic spread of tumor.

Clinical approaches towards induction of innate immunity for therapeutic anti-cancer activity

KIR/KIR-ligand (KIR/KIR-L) mismatch in BMT

The graft-vs.-leukemia (GVL) effect, in which anti-tumor immunity is conferred to a patient via bone-marrow transplant, may be among the first examples of a successful immunotherapy. The effect, noted in animal models, and then in clinical BMT data, as an association between graft vs. host disease (GVHD) and lower leukemic relapse rates, began efforts to promote GVL without GVHD. The ideal cells to achieve GVL would recognize the patient's normal tissues as "self" yet recognize leukemic cells as "non-self" and target them for destruction. Allogeneic donor T cells are capable of eliminating neoplastic cells (GVL-effect) but can also mediate GVHD as they may be activated by MHC presenting recipient peptides that are "foreign" to the donor T cells. In contrast, donor NK cells can be activated by NK-activating ligands on leukemic cells but this activation needs to circumvent inhibition via the inhibitory KIR receptors. This principle was initially exploited in the clinical setting of BMT for acute myeloid leukemia. Patients receiving transplants with a KIR/KIR-L incompatibility (circumventing NK inhibition) experienced a 60% event free survival at 5 years vs. 5% for patients in which NK cell inhibition was induced on the basis of KIR/KIR-L match ($p<0.0005$)[17].

Administration of IL2 alone to stimulate anti-tumor responses

IL2 is a cytokine secreted by $CD4^+$ and other immune cells and has various effects on distinct immune cells. Exposure of human peripheral blood mononuclear cells (PBMCs) to IL2 generates lymphokine-activated killer cells capable of spontaneous lysis of tumor cells *in vitro*. Administration of this cytokine to melanoma and renal cell carcinoma patients has been one of the earliest successful applications of an immunotherapy[18]. While most evidence suggests that the anti-tumor effects of IL2 are mediated by antigen-specific T cells, a role for activation of innate immunity cannot be excluded. Even so, the activation of innate immune cells with IL2 may not provide the level of specific tumor recognition that is desired in order to selectively attack tumor cells. Added tumor specificity has become possible by incorporating the strategy of tumor-specific monoclonal antibody.

Tumor-selective attack by innate immune effector cells facilitated by tumor-reactive monoclonal antibodies

A variety of established laboratory techniques now allow for the creation human mAbs against a wide variety of epitopes[19]. Many clinically effective tumor specific mAbs induce direct tumor cell destruction involving innate immune mechanisms; some work via complement dependent cytotoxicity, while most involve innate effector cells via ADCC. Table 1 provides examples of tumor-reactive mAbs with proven antitumor efficacy, that work, at least in part, via these innate immune pathways. In ADCC, the antigen specific (Fab

portion) of the antibody binds a tumor cell surface epitope while the Fc portion of the antibody recruits immune cells bearing an activating Fc-receptor (mainly NK cells, macrophages and neutrophils).

While some mAbs show clinical benefit as single agent therapy, additional benefit is obtained when mAbs are combined with immune-stimulation. NK cells, macrophages, and neutrophils (the main effector cells mediating ADCC) can be augmented to improve outcome.

Enhancing antibody-directed innate immunity to improve outcome: an example combining an anti-GD2 mAb, IL2, and GM-CSF for pediatric high-risk neuroblastoma

The GD2 disialoganglioside is expressed on neuroectodermal tumors, including melanoma and neuroblastoma[20–23], but not on normal tissues other than low-level expression on certain neurons and melanocytes. Clinical testing has included the 3F8 and 14.G2a murine mAbs, the chimeric 14.18 (ch14.18) mAb and its modified humanized form (hu14.18K322A)[24–34]. *In vitro*, anti-GD2 mAbs can mediate ADCC against $GD2^+$ tumor cells[35, 36]. PBMCs from patients treated with IL2 *in vivo* mediate enhanced ADCC with the 14.G2a, ch14.18 and 3F8 mAbs[37] suggesting that anti-GD2 mAbs may provide benefit in patients receiving IL2. In initial phase-I/II trials, most patients with measureable disease showed no effect in response to 14.G2a or ch14.18 plus IL2[38, 39]. This clinical experience, coupled with preclinical data showing better results of mAb based immunotherapy in smaller, less-established disease[40], suggested that this approach should be tested in patients with minimal residual disease (MRD), such as patients in remission but at high risk for recurrence. This approach was tested in pilot studies[41, 42] for children with high-risk neuroblastoma following intensive chemotherapy, surgery, radiation therapy, autologous stem cell transplant (ASCT) and cis-retinoic acid (CRA); historically such children had only a ~40% chance of event-free survival (EFS)[43]. In a small pilot study for children with high-risk neuroblastoma following ASCT, IL2 was combined with GM-CSF (to enhance ADCC by NK cells as well as macrophages and neutrophils) and with ch14.18[44, 45]. Toxicity was acceptable; 2 year overall survival was ~75%, better than historical controls[41]. These results led to a Phase III randomized trial of ch14.18 + GM-CSF + IL2 + CRA (immunotherapy) vs. CRA. With a median follow-up time of ~2 years, the event-free-survival (EFS) at 2 years was 66% for immunotherapy vs. 46% for CRA ($p = 0.0115$). The overall survival at 2 and 3 years, respectively was 86% and 79% for immunotherapy, vs. 75% and 63% for CRA ($p = 0.0223$)[46]. Despite the absence of a control arm of cytokines alone, this Phase-III trial provided evidence that a combination of anti-cancer mAb with cytokines is an effective anti-tumor treatment, and is now the standard of care regimen (and FDA approved) for children with high-risk neuroblastoma.

Future applicability to other cancers treated with other antibodies

To date, published clinical trials of regimens adding IL2 or GM-CSF to treatment with other ADCC-inducing mAbs (ie: Rituximab, Trastuzumab, see Table 1) have not shown any benefit over treatment with the mAb alone[47–49]. However, these published studies have focused on treatment for refractory or relapsed measureable disease. The study of combination immunotherapy in high-risk neuroblastoma suggests that the efficacy of

ADCC-inducing mAbs may be best seen when combined with agents that augment ADCC and when tested in the adjuvant or minimal residual disease setting[46].

In applying combination immunotherapy, with ADCC-acting mAb and immunostimulatory agents, the challenges will be to identify: 1) which combinations of immunotherapies work best together and in what order; 2) how to combine these with “conventional therapies”; 3) when in the overall clinical treatment course are these approaches best applied; and 4) how to integrate patient-specific genetic information (pertaining to the tumor and to each patient’s immune capabilities) into the design and selection of regimens for individual patients.

Next-generation mAb-based agents

Just as tumor-reactive mAbs can target innate immune cells with Fc receptors to tumor cells, next-generation agents involving tumor-reactive mAbs can use other effector cell triggering structures to bridge effector cells to tumor cells and facilitate their destruction.

Bifunctional mAbs—Bifunctional antibodies can involve chemical or genetic linkage of tumor-reactive mAbs to other mAbs that bind triggering structures on effector cells. Catumaxomab links a mAb against epithelial cell adhesion molecule (EpCAM, which is expressed on ovarian cancer as well as several other epithelial cancers) to a triggering anti-CD3 mAb that activates T-cells regardless of their T-cell receptor specificity. This agent is approved as an intraperitoneal therapeutic for ovarian cancer in Europe[50]. Similar in intent is Blinatumomab, a bifunctional agent that links a single chain Fab- variable (scFv) fragment against CD-19 (on pre-B leukemia) to a scFV against CD-3. This agent has shown dramatic success in treating refractory pre-B acute lymphoblastic leukemia, and has recently been approved by FDA [51, 52].

Immunocytokines—Immunocytokines (ICs) are fusion proteins that are functionally similar to bifunctional mAbs. ICs are created by linking a tumor-reactive mAb to a cytokine that activates effector cells (rather than to a separate mAb that activates effector cells). Selective delivery of the broadly acting cytokine to the tumor makes ICs more potent in tumor-bearing mice than comparable amounts of the same mAb and cytokine, given separately yet simultaneously[40, 53, 54]. In a preclinical study of an IC specific for the CD20 antigen on malignant B cells, the *in vivo* anti-tumor efficacy of the IC was far greater than the efficacy seen using 25 fold more mAb and IL2 given simultaneously[55]. In addition, ICs can function in ways that are not possible by conventional mAbs. For example, when an anti-GD2 IC (consisting of an anti-GD2 mAb coupled to IL2, designated hu14.18-IL2) binds to a GD2+ tumor cell, it essentially coats the tumor cell’s surface with IL2, allowing effector cells to use their IL2 receptors (IL2Rs) to bind to IL2-coated tumor cells. This leads to induction of an IL2R-facilitated activated immune synapse with the NK and tumor cells[56, 57]. Conceptually, this means that these ICs are bifunctional agents (for NK-mediated anti-tumor effects) with a similar mechanism to bifunctional mAbs that link an anti-tumor mAb to an activating receptor (like-CD3) on a T cell, thus binding tumor cells to activated T cells (like blinatumomab)[51, 52].

These bifunctional agents (like ICs) are “off the shelf” therapeutics that are schematically analogous to the chimeric antigen receptor modified T-cell (CART) approach; both use mAb recognition of tumors to bind effector cells to the cancer and induce downstream immune activation, resulting in tumor cytolysis. Hu14.18-IL2 has shown significant clinical activity in children with neuroblastoma[58]. A growing number of widely diverse IC’s, varying in their antigenic targets, cytokine conjugate, and structure are currently being evaluated clinically[59].

Promising area of translational research: understanding the relationships between KIR/KIR-L and response to ADCC acting immunotherapy

Given the important role of KIR/KIR-ligand (KIR/KIR-L) relationships in the setting of allogeneic BMT, it is conceivable that these same relationships might influence clinical efficacy in the setting of NK-mediated ADCC using tumor-reactive mAbs. This was evaluated using data from a phase-II clinical trial of the hu14.18-IL2 IC in patients with relapsed/refractory neuroblastoma. In this small study, all patients that showed clinical benefit in response to hu14.18-IL2 lacked an HLA ligand for at least one of their KIR genes[60]. Subsequent analyses from a larger trial of anti-GD2 mAb for neuroblastoma confirmed the importance of KIR/KIR-L mismatch in this *in vivo* ADCC clinical effect[61]. In the case of lymphoma patients treated with rituximab (anti-CD20 mAb), clinical associations between immunotherapeutic outcome and KIR/KIR-L status are also seen[62, 63]. Even so, some of the associations seen for KIR/KIR-L associations in rituximab-treated patients are somewhat distinct from results for analyses in neuroblastoma patients receiving anti-GD2 mAb and cytokines[60, 61] or for recipients of allo-BMT[17, 64–70]. These results suggest that the KIR/KIR-L relationships with outcome may be different for different forms of immunotherapy or for different forms of cancer.

As the relationships between KIR/KIR-L status and treatment outcome become clearer, these relationships may help in clinical decision-making. “Favorable” vs. “unfavorable” KIR/KIR-L status (however they may be defined based on disease and treatment specific data) might be used prospectively for: 1) clinical trial subset analyses; 2) eligibility criteria for entry to certain trials for clinical immunotherapy; or 3) determination of patients with an “unfavorable” KIR-ligand status who might best benefit from “KIR- blockade” via the addition of a separate anti-inhibitory-KIR mAb (such as Lirilumab) to their ADCC-therapeutic regimen. Lirilumab is an antagonist mAb reactive with inhibitory KIRs 2DL1, -L2, -L3; it augments NK cell cytotoxicity and may augment efficacy of mAbs acting through ADCC[71]. It is currently being evaluated in a number of phase I clinical trials for advanced solid tumors, as part of checkpoint blockade strategies[72].

Other approaches towards NK cell stimulation

The response of NK cells to various interleukins is a feature of NK cells now being employed in various treatment strategies. *In vivo* NK cell activation is achievable with the administration of interleukin-2 (IL2) or Interleukin-15 (IL15)[73].

Macrophages as targets for induction of innate anti-tumor immune responses

Manipulation of monocytes/macrophages

In the context of cancer immunotherapy, the capability of macrophages to take on either a pro- or anti-tumoral function presents distinct therapeutic targets: activation of M1 (anti-tumoral) macrophages and inhibition (or repolarization to M1) of M2 (pro-tumoral) macrophages. Macrophage colony stimulating factor (M-CSF) acts on macrophages via an M-CSF receptor. Of interest, M-CSF has been implicated in polarization of macrophages towards the M2 phenotype. In some cancers (including breast, ovarian epithelial, endometrioid, papillary renal cell carcinoma), M-CSF expression levels have been associated with higher tumor grade, more metastases, and poor prognoses. A number of M-CSF and M-CSF receptor inhibitors have been evaluated in xenograft models, with highly variable results dependent on tumor model and inhibitor. While results were not striking when these were given alone, augmentation of the anti-tumor effects of radio or chemotherapy have been reported with their use[75]. Currently, human trials for advanced solid tumors are being conducted with 2 separate anti-M-CSF receptor mAbs [IMC-CS4 (fully human IgG1 mAb)[76] and RG7155 (humanized IgG1 mAb)[77]] and a small molecule receptor tyrosine kinase inhibitor (PLX 3397)[78–84].

Methods of activating M1-macrophages: TLR agonism

A potent, evolutionarily conserved innate immune mechanism to rapidly and robustly activate monocytes/macrophages is through the toll-like receptor (TLR) system. TLRs exist in a variety of locations on and in macrophages, and are designed to recognize and respond to molecular patterns shared by pathogenic organisms. While some of the TLRs are internally located (well-suited to detect viral components), cell surface TLRs have been implicated in the immune response to cancer. Specific molecular patterns, characteristic of tumor cells as they are killed via radio- or chemotherapy, are recognized by TLRs and can potentially form a bridge between the innate and adaptive immune responses[85]. Preclinical approaches have demonstrated how activation of macrophages with anti-CD40 agonist mAb, combined with TLR activation can induce augmented M1 macrophage function, augmented antitumor effects[86, 87] and enhanced *in vivo* tumor-reactive mAb induced antitumor effects[88].

Clinical approaches towards TLR agonism

A cell wall component of Bacillus Calmette-Guerin, an activator of TLR2 and TLR4, has been approved by FDA for adult bladder cancer. Imiquimod, a TLR7 agonist, has been approved by FDA for the treatment of basal cell carcinoma. A number of additional TLR4, -5, -7, and -8 agonists are also being investigated[89].

Clinical strategy towards macrophage activation in osteosarcoma

In osteosarcoma the benefit of surgical resection and adjuvant chemotherapy is still associated with a 30–40% rate of relapse, often presenting as chemo-resistant pulmonary metastases[90]. Muramyl tripeptide phosphatidylethanolamine (MTP-PE) is a structural

component of mycobacterium, phagocytosed by monocytes and macrophages. Through direct cell stimulation, MTP-PE polarizes macrophages towards tumoricidal (M1) activity via up-regulation of TNF, IL1, IL6, IL8, and monocyte chemotactic activating factor (MCAF)[91]. MTP-PE's effect on macrophages is not affected *in vivo* by concomitant administration of cisplatin, high-dose methotrexate, cyclophosphamide, or doxorubicin[91]. This agent was not found to appreciably augment the toxicities observed with these chemotherapies. A liposomal encapsulation of MTP-PE was used as part of a multimodality treatment protocol with clinical benefit in pediatric osteosarcoma, conferring an overall survival benefit at 6 years post treatment in one of the treated cohorts ($p=0.03$)[92].

Antitumor activity of macrophage targeted immunotherapy

In vivo treatment with anti-CD40 agonist mAbs activates macrophages to release IFN-γ IL12 and nitric oxide (NO), mediate destruction of tumor targets via apoptosis *in vitro*, and up-regulate TLRs[86, 93–95]. Further, anti-CD40 can synergize with the TLR9 agonist, CpG[96], and with the TLR4 agonist, Monophosphoryl Lipid A (MPL)[87], in activating macrophages and inducing anti-tumor effects *in vitro* and *in vivo*. Certain chemotherapies can synergize with anti-CD40 and CpG to change tumor-associated macrophages from pro-tumor M2 phenotype to anti-tumor M1 phenotype, resulting in anti-tumor effects in murine melanoma and neuroblastoma models[97]. Mouse studies confirmed that the anti-tumor actions of anti-CD40 can involve macrophages[98]. In humans, these regimens have demonstrated clinical benefit in some patients with pancreatic cancer[98].

Induction of innate immunity as a bridge to adaptive immunity

Many tumors, especially those arising under carcinogenic pressures, exhibit potent tumor specific antigens. Scrutiny of the tumor microenvironment in a subset of patients reveals an adaptive T cell immune response specific for these tumor antigens, but unable to eradicate the cancer[99]. In this subset of patients, innate immune mechanisms were able to recognize the cancer and active ineffective adaptive immune elements. Strategies aimed at enhancement of the impaired adaptive immune systems in these patients (such as high dose-IL2 and/or checkpoint blockade) have been able to restore potent anti-tumor effects and long lasting immunity.

In other patients, the tumor microenvironment is devoid of infiltrating immune cells and lacks signs of immune activation[99]. As this subset of patients shows less evidence of innate immune engagement, strategies must be aimed at enhancing the innate immune recognition of cancer[99] and promoting an innate immune response against cancer before engaging the strategies designed to enhance the adaptive immune system.

Future direction: potential integration of innate and adaptive immune responses for the treatment of advanced cancer

Many immunotherapeutic strategies enhancing distinct aspects of the immune system are showing clinical promise. Moving forward, it will be important to identify ways in which strategies that engage the innate immune system can be augmented and combined with adaptive immune enhancement. We believe that the following proposed generalized

approach, or variations based on this strategy, warrant testing as they may prove to be effective and applicable to many cancers. Treatment might be initiated with a mAb-based agent (or agents in combination). These can be mAbs, their genetically engineered derivatives, bifunctional agents or ICs, as long as they are able to recognize cancer-selective cell surface targets (molecules that are overexpressed by the cancer, but with very low expression on normal tissues), and initiate cell-mediated anti-tumor activity[100, 101]. Tumor destruction can be enhanced in this setting by the provision of agents (like IL2, IL15 or GM-CSF; alone or as ICs) to activate innate effector cells, such as NK cells, neutrophils and macrophages that mediate ADCC[46, 102, 103]. This process can also enable the mAb-coated dying tumor cells to be taken up by antigen presenting cells (APCs), thus serving as an endogenous autologous tumor vaccine[104]. This should induce or augment the anti-tumor adaptive response (that had not yet been effective) by more potently immunizing the patients with their own set of unique tumor antigens[3, 105–107]. Once an adaptive response is initiated, the addition of agents that expand the growing innate and adaptive responses should prove synergistic. These approaches include: 1) providing stimulation via immune potentiating "agonist" antibodies (such as anti CD40 or anti-CD137)[98, 108, 109]; 2) taking "the brakes off" the innate and adaptive immune response by blocking inhibitory checkpoints (PD1, CTLA4, KIR)[71, 110–113]; or 3) interfering with inhibitory cells [such as regulatory T cells or myeloid derived suppressor cells][114]. The challenge for applying this type of combined approach involves selecting the right available agents, for the right patients, and applying this strategy at the optimal time in their overall cancer treatment.

Acknowledgments

This work was supported, in part, by National Institutes of Health Grants CA032685, CA87025, CA166105, CA14520, GM067386, and grants from the Midwest Athletes for Childhood Cancer Fund, The Crawdaddy Foundation, The Evan Dunbar Foundation, Alex's Lemonade Stand Foundation, Hyundai Hope on Wheels, the University of Wisconsin-Madison Institute for Clinical and Translational Research Grant 1TL1RR025013-01 and by a Stand Up To Cancer – St. Baldrick's Pediatric Dream Team Translational Research Grant (SU2C-AACR-DT1113). Stand Up To Cancer is a program of the Entertainment Industry Foundation administered by the American Association for Cancer Research. JLG is supported, in part, by a grant from the Howard Hughes Medical Institute Medical Fellows Research Program.

References

1. Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ, et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood. 1990; 75:555–62.
2. Couzin-Frankel J. Breakthrough of the year 2013. Cancer immunotherapy. Science. 2013; 342:1432–3. [PubMed: 24357284]
3. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science. 2011; 331:1565–70. [PubMed: 21436444]
4. Whiteside TL. Inhibiting the inhibitors: evaluating agents targeting cancer immunosuppression. Expert Opin Biol Ther. 2010; 10:1019–35. [PubMed: 20415597]
5. Mackall CL, Fleisher TA, Brown MR, Magrath IT, Shad AT, Horowitz ME, et al. Lymphocyte depletion during treatment with intensive chemotherapy for cancer. Blood. 1994; 84:2221–8. [PubMed: 7919339]
6. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. Annu Rev Immunol. 2004; 22:329–60. [PubMed: 15032581]
7. Kim S, Iizuka K, Aguila HL, Weissman IL, Yokoyama WM. In vivo natural killer cell activities revealed by natural killer cell-deficient mice. Proc Natl Acad Sci U S A. 2000; 97:2731–6. [PubMed: 10694580]

8. Jensen HK, Donskov F, Marcussen N, Nordsmark M, Lundbeck F, von der Maase H. Presence of intratumoral neutrophils is an independent prognostic factor in localized renal cell carcinoma. J Clin Oncol. 2009; 27:4709–17. [PubMed: 19720929]
9. Wislez M, Rabbe N, Marchal J, Milleron B, Crestani B, Mayaud C, et al. Hepatocyte growth factor production by neutrophils infiltrating bronchioloalveolar subtype pulmonary adenocarcinoma: role in tumor progression and death. Cancer Res. 2003; 63:1405–12. [PubMed: 12649206]
10. Bellocq A, Antoine M, Flahault A, Philippe C, Crestani B, Bernaudin JF, et al. Neutrophil alveolitis in bronchioloalveolar carcinoma: induction by tumor-derived interleukin-8 and relation to clinical outcome. Am J Pathol. 1998; 152:83–92. [PubMed: 9422526]
11. Gregory AD, Houghton AM. Tumor-associated neutrophils: new targets for cancer therapy. Cancer Res. 2011; 71:2411–6. [PubMed: 21427354]
12. Houghton AM, Rzymkiewicz DM, Ji H, Gregory AD, Egea EE, Metz HE, et al. Neutrophil elastase-mediated degradation of IRS-1 accelerates lung tumor growth. Nat Med. 2010; 16:219–23. [PubMed: 20081861]
13. Di Carlo E, Forni G, Lollini P, Colombo MP, Modesti A, Musiani P. The intriguing role of polymorphonuclear neutrophils in antitumor reactions. Blood. 2001; 97:339–45. [PubMed: 11154206]
14. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, et al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: “N1” versus “N2” TAN. Cancer Cell. 2009; 16:183–94. [PubMed: 19732719]
15. Cheung NK, Cheung IY, Kushner BH, Ostrovnaya I, Chamberlain E, Kramer K, et al. Murine anti-GD2 monoclonal antibody 3F8 combined with granulocyte-macrophage colony-stimulating factor and 13-cis-retinoic acid in high-risk patients with stage 4 neuroblastoma in first remission. J Clin Oncol. 2012; 30:3264–70. [PubMed: 22869886]
16. Mantovani A, Allavena P, Sica A. Tumour-associated macrophages as a prototypic type II polarised phagocyte population: role in tumour progression. Eur J Cancer. 2004; 40:1660–7. [PubMed: 15251154]
17. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002; 295:2097–100. [PubMed: 11896281]
18. Rosenberg SA. IL-2: the first effective immunotherapy for human cancer. J Immunol. 2014; 192:5451–8. [PubMed: 24907378]
19. Nelson AL, Dhimolea E, Reichert JM. Development trends for human monoclonal antibody therapeutics. Nat Rev Drug Discov. 2010; 9:767–74. [PubMed: 20811384]
20. Mujoo K, Cheresh DA, Yang HM, Reisfeld RA. Disialoganglioside GD2 on human neuroblastoma cells: target antigen for monoclonal antibody-mediated cytolysis and suppression of tumor growth. Cancer Res. 1987; 47:1098–104. [PubMed: 3100030]
21. Cheung NK, Saarinen UM, Neely JE, Landmeier B, Donovan D, Coccia PF. Monoclonal antibodies to a glycolipid antigen on human neuroblastoma cells. Cancer Res. 1985; 45:2642–9. [PubMed: 2580625]
22. Schulz G, Cheresh DA, Varki NM, Yu A, Staffileno LK, Reisfeld RA. Detection of ganglioside GD2 in tumor tissues and sera of neuroblastoma patients. Cancer Res. 1984; 44:5914–20. [PubMed: 6498849]
23. Kramer K, Gerald WL, Kushner BH, Larson SM, Hameed M, Cheung NK. Disialoganglioside G(D2) loss following monoclonal antibody therapy is rare in neuroblastoma. Clin Cancer Res. 1998; 4:2135–9. [PubMed: 9748131]
24. Cheung NK, Lazarus H, Miraldi FD, Abramowsky CR, Kallick S, Saarinen UM, et al. Ganglioside GD2 specific monoclonal antibody 3F8: a phase I study in patients with neuroblastoma and malignant melanoma. J Clin Oncol. 1987; 5:1430–40. [PubMed: 3625258]
25. Cheung NK, Kushner BH, Cheung IY, Kramer K, Canete A, Gerald W, et al. Anti-G(D2) antibody treatment of minimal residual stage 4 neuroblastoma diagnosed at more than 1 year of age. J Clin Oncol. 1998; 16:3053–60. [PubMed: 9738575]

26. Handgretinger R, Baader P, Dopfer R, Klingebiel T, Reuland P, Treuner J, et al. A phase I study of neuroblastoma with the anti-ganglioside GD2 antibody 14.G2a. Cancer Immunol Immunother. 1992; 35:199–204. [PubMed: 1638557]
27. Yu AL, Uttenreuther-Fischer MM, Huang CS, Tsui CC, Gillies SD, Reisfeld RA, et al. Phase I trial of a human-mouse chimeric anti-disialoganglioside monoclonal antibody ch14.18 in patients with refractory neuroblastoma and osteosarcoma. J Clin Oncol. 1998; 16:2169–80. [PubMed: 9626218]
28. Handgretinger R, Anderson K, Lang P, Dopfer R, Klingebiel T, Schrappe M, et al. A phase I study of human/mouse chimeric antiganglioside GD2 antibody ch14.18 in patients with neuroblastoma. Eur J Cancer. 1995; 31A:261–7. [PubMed: 7718335]
29. Murray JL, Cunningham JE, Brewer H, Mujoo K, Zukiwski AA, Podoloff DA, et al. Phase I trial of murine monoclonal antibody 14G2a administered by prolonged intravenous infusion in patients with neuroectodermal tumors. J Clin Oncol. 1994; 12:184–93. [PubMed: 8270976]
30. Saleh MN, Khazaeli MB, Wheeler RH, Allen L, Tilden AB, Grizzle W, et al. Phase I trial of the chimeric anti-GD2 monoclonal antibody ch14.18 in patients with malignant melanoma. Hum Antibodies Hybridomas. 1992; 3:19–24. [PubMed: 1576319]
31. Saleh MN, Khazaeli MB, Wheeler RH, Dropcho E, Liu T, Urist M, et al. Phase I trial of the murine monoclonal anti-GD2 antibody 14G2a in metastatic melanoma. Cancer Res. 1992; 52:4342–7. [PubMed: 1643631]
32. Yeh SD, Larson SM, Burch L, Kushner BH, Laquaglia M, Finn R, et al. Radioimmunodetection of neuroblastoma with iodine-131-3F8: correlation with biopsy, iodine-131-metaiodobenzylguanidine and standard diagnostic modalities. J Nucl Med. 1991; 32:769–76. [PubMed: 1902508]
33. Navid F, Sondel PM, Barfield R, Shulkin BL, Kaufman RA, Allay JA, et al. Phase I trial of a novel anti-GD2 monoclonal antibody, Hu14.18K322A, designed to decrease toxicity in children with refractory or recurrent neuroblastoma. J Clin Oncol. 2014; 32:1445–52. [PubMed: 24711551]
34. Reuland P, Geiger L, Thelen MH, Handgretinger R, Haase B, Muller-Schauenburg W, et al. Follow-up in neuroblastoma: comparison of metaiodobenzylguanidine and a chimeric anti-GD2 antibody for detection of tumor relapse and therapy response. J Pediatr Hematol Oncol. 2001; 23:437–42. [PubMed: 11878578]
35. Munn DH, Cheung NK. Interleukin-2 enhancement of monoclonal antibody-mediated cellular cytotoxicity against human melanoma. Cancer Res. 1987; 47:6600–5. [PubMed: 3499978]
36. Barker E, Reisfeld RA. A mechanism for neutrophil-mediated lysis of human neuroblastoma cells. Cancer Res. 1993; 53:362–7. [PubMed: 8417829]
37. Hank JA, Robinson RR, Surfus J, Mueller BM, Reisfeld RA, Cheung NK, et al. Augmentation of antibody dependent cell mediated cytotoxicity following in vivo therapy with recombinant interleukin 2. Cancer Res. 1990; 50:5234–9. [PubMed: 2386933]
38. Frost JD, Hank JA, Reaman GH, Frierdich S, Seeger RC, Gan J, et al. A phase I/IB trial of murine monoclonal anti-GD2 antibody 14.G2a plus interleukin-2 in children with refractory neuroblastoma: a report of the Children's Cancer Group. Cancer. 1997; 80:317–33. [PubMed: 9217046]
39. Albertini MR, Hank JA, Schiller JH, Khorsand M, Borchert AA, Gan J, et al. Phase IB trial of chimeric antidisialoganglioside antibody plus interleukin 2 for melanoma patients. Clin Cancer Res. 1997; 3:1277–88. [PubMed: 9815810]
40. Neal ZC, Yang JC, Rakhmilevich AL, Buhtoiarov IN, Lum HE, Imboden M, et al. Enhanced activity of hu14.18-IL2 immunocytokine against murine NXS2 neuroblastoma when combined with interleukin 2 therapy. Clin Cancer Res. 2004; 10:4839–47. [PubMed: 15269160]
41. Gilman AL, Ozkaynak MF, Matthay KK, Krailo M, Yu AL, Gan J, et al. Phase I study of ch14.18 with granulocyte-macrophage colony-stimulating factor and interleukin-2 in children with neuroblastoma after autologous bone marrow transplantation or stem-cell rescue: a report from the Children's Oncology Group. J Clin Oncol. 2009; 27:85–91. [PubMed: 19047298]
42. Ozkaynak MF, Sondel PM, Krailo MD, Gan J, Javorsky B, Reisfeld RA, et al. Phase I study of chimeric human/murine anti-ganglioside G(D2) monoclonal antibody (ch14.18) with granulocyte-macrophage colony-stimulating factor in children with neuroblastoma immediately after

hematopoietic stem-cell transplantation: a Children's Cancer Group Study. J Clin Oncol. 2000; 18:4077–85. [PubMed: 11118469]

43. Matthay KK, Villablanca JG, Seeger RC, Stram DO, Harris RE, Ramsay NK, et al. Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. Children's Cancer Group. N Engl J Med. 1999; 341:1165–73. [PubMed: 10519894]
44. Kushner BH, Kramer K, Cheung NK. Phase II trial of the anti-G(D2) monoclonal antibody 3F8 and granulocyte-macrophage colony-stimulating factor for neuroblastoma. J Clin Oncol. 2001; 19:4189–94. [PubMed: 11709561]
45. Cheung IY, Lo Piccolo MS, Kushner BH, Cheung NK. Early molecular response of marrow disease to biologic therapy is highly prognostic in neuroblastoma. J Clin Oncol. 2003; 21:3853–8. [PubMed: 14551304]
46. Yu AL, Gilman AL, Ozkaynak MF, London WB, Kreissman SG, Chen HX, et al. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. N Engl J Med. 2010; 363:1324–34. [PubMed: 20879881]
47. Hjelm Skog A, Ragnhammar P, Fagerberg J, Frodin J, Goldinger M, Koldestam H, et al. Clinical effects of monoclonal antibody 17-1A combined with granulocyte/macrophage-colony-stimulating factor and interleukin-2 for treatment of patients with advanced colorectal carcinoma. Cancer Immunol Immunother. 1999; 48:463–70. [PubMed: 10550551]
48. Khan KD, Emmanouilides C, Benson DM Jr, Hurst D, Garcia P, Michelson G, et al. A phase 2 study of rituximab in combination with recombinant interleukin-2 for rituximab-refractory indolent non-Hodgkin's lymphoma. Clin Cancer Res. 2006; 12:7046–53. [PubMed: 17145827]
49. Mani A, Roda J, Young D, Caligiuri MA, Fleming GF, Kaufman P, et al. A phase II trial of trastuzumab in combination with low-dose interleukin-2 (IL-2) in patients (PTS) with metastatic breast cancer (MBC) who have previously failed trastuzumab. Breast Cancer Res Treat. 2009; 117:83–9. [PubMed: 19051009]
50. Linke R, Klein A, Seimetz D. Catumaxomab: clinical development and future directions. MAbs. 2010; 2:129–36. [PubMed: 20190561]
51. Schlegel P, Lang P, Zugmaier G, Ebinger M, Kreyenberg H, Witte KE, et al. Pediatric posttransplant relapsed/refractory B-precursor acute lymphoblastic leukemia shows durable remission by therapy with the T-cell engaging bispecific antibody blinatumomab. Haematologica. 2014; 99:1212–9. [PubMed: 24727818]
52. Moore PA, Zhang W, Rainey GJ, Burke S, Li H, Huang L, et al. Application of dual affinity retargeting molecules to achieve optimal redirected T-cell killing of B-cell lymphoma. Blood. 2011; 117:4542–51. [PubMed: 21300981]
53. Imboden M, Murphy KR, Rakhmilevich AL, Neal ZC, Xiang R, Reisfeld RA, et al. The level of MHC class I expression on murine adenocarcinoma can change the antitumor effector mechanism of immunocytokine therapy. Cancer Res. 2001; 61:1500–7. [PubMed: 11245457]
54. Lode HN, Xiang R, Dreier T, Varki NM, Gillies SD, Reisfeld RA. Natural killer cell-mediated eradication of neuroblastoma metastases to bone marrow by targeted interleukin-2 therapy. Blood. 1998; 91:1706–15. [PubMed: 9473237]
55. Gillies SD, Lan Y, Williams S, Carr F, Forman S, Raubitschek A, et al. An anti-CD20-IL-2 immunocytokine is highly efficacious in a SCID mouse model of established human B lymphoma. Blood. 2005; 105:3972–8. [PubMed: 15692062]
56. Buhtoiarov IN, Neal ZC, Gan J, Buhtoiarova TN, Patankar MS, Gubbels JA, et al. Differential internalization of hu14.18-IL2 immunocytokine by NK and tumor cell: impact on conjugation, cytotoxicity, and targeting. J Leukoc Biol. 2011; 89:625–38. [PubMed: 21248148]
57. Gubbels JA, Gadbaw B, Buhtoiarov IN, Horibata S, Kapur AK, Patel D, et al. Ab-IL2 fusion proteins mediate NK cell immune synapse formation by polarizing CD25 to the target cell-effector cell interface. Cancer Immunol Immunother. 2011; 60:1789–800. [PubMed: 21792658]
58. Shusterman S, London WB, Gillies SD, Hank JA, Voss SD, Seeger RC, et al. Antitumor activity of hu14.18-IL2 in patients with relapsed/refractory neuroblastoma: a Children's Oncology Group (COG) phase II study. J Clin Oncol. 2010; 28:4969–75. [PubMed: 20921469]

59. List T, Neri D. Immunocytokines: a review of molecules in clinical development for cancer therapy. Clin Pharmacol. 2013; 5:29–45. [PubMed: 23990735]

60. Delgado DC, Hank JA, Kolesar J, Lorentzen D, Gan J, Seo S, et al. Genotypes of NK cell KIR receptors, their ligands, and Fcgamma receptors in the response of neuroblastoma patients to Hu14.18-IL2 immunotherapy. Cancer Res. 2010; 70:9554–61. [PubMed: 20935224]

61. Tarek N, Le Luduec JB, Gallagher MM, Zheng J, Venstrom JM, Chamberlain E, et al. Unlicensed NK cells target neuroblastoma following anti-GD2 antibody treatment. J Clin Invest. 2012; 122:3260–70. [PubMed: 22863621]

62. Wilson MJ, Torkar M, Haude A, Milne S, Jones T, Sheer D, et al. Plasticity in the organization and sequences of human KIR/ILT gene families. Proc Natl Acad Sci U S A. 2000; 97:4778–83. [PubMed: 10781084]

63. Uhrberg M, Valiante NM, Shum BP, Shilling HG, Lienert-Weidenbach K, Corliss B, et al. Human diversity in killer cell inhibitory receptor genes. Immunity. 1997; 7:753–63. [PubMed: 9430221]

64. Ruggeri L, Mancusi A, Burchielli E, Capanni M, Carotti A, Aloisi T, et al. NK cell alloreactivity and allogeneic hematopoietic stem cell transplantation. Blood Cells Mol Dis. 2008; 40:84–90. [PubMed: 17964828]

65. Leung W, Iyengar R, Turner V, Lang P, Bader P, Conn P, et al. Determinants of antileukemia effects of allogeneic NK cells. J Immunol. 2004; 172:644–50. [PubMed: 14688377]

66. Leung W, Handgretinger R, Iyengar R, Turner V, Holladay MS, Hale GA. Inhibitory KIR-HLA receptor-ligand mismatch in autologous haematopoietic stem cell transplantation for solid tumour and lymphoma. Br J Cancer. 2007; 97:539–42. [PubMed: 17667923]

67. Hsu KC, Keever-Taylor CA, Wilton A, Pinto C, Heller G, Arkun K, et al. Improved outcome in HLA-identical sibling hematopoietic stem-cell transplantation for acute myelogenous leukemia predicted by KIR and HLA genotypes. Blood. 2005; 105:4878–84. [PubMed: 15731175]

68. Venstrom JM, Zheng J, Noor N, Danis KE, Yeh AW, Cheung IY, et al. KIR and HLA genotypes are associated with disease progression and survival following autologous hematopoietic stem cell transplantation for high-risk neuroblastoma. Clin Cancer Res. 2009; 15:7330–4. [PubMed: 19934297]

69. Cooley S, Weisdorf DJ, Guethlein LA, Klein JP, Wang T, Le CT, et al. Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia. Blood. 2010; 116:2411–9. [PubMed: 20581313]

70. Pende D, Marcenaro S, Falco M, Martini S, Bernardo ME, Montagna D, et al. Anti-leukemia activity of alloreactive NK cells in KIR ligand-mismatched haploidentical HSCT for pediatric patients: evaluation of the functional role of activating KIR and redefinition of inhibitory KIR specificity. Blood. 2009; 113:3119–29. [PubMed: 18945967]

71. Kohrt HE, Thielens A, Marabelle A, Sagiv-Barfi I, Sola C, Chanuc F, et al. Anti-KIR antibody enhancement of anti-lymphoma activity of natural killer cells as monotherapy and in combination with anti-CD20 antibodies. Blood. 2014; 123:678–86. [PubMed: 24326534]

72. Aranda F, Vacchelli E, Eggermont A, Galon J, Fridman WH, Zitvogel L, et al. Trial Watch: Immunostimulatory monoclonal antibodies in cancer therapy. Oncoimmunology. 2014; 3:e27297. [PubMed: 24701370]

73. Conlon KC, Lugli E, Welles HC, Rosenberg SA, Fojo AT, Morris JC, et al. Redistribution, Hyperproliferation, Activation of Natural Killer Cells and CD8 T Cells, and Cytokine Production During First-in-Human Clinical Trial of Recombinant Human Interleukin-15 in Patients With Cancer. J Clin Oncol. 2014

74. Huang S, Mills L, Mian B, Tellez C, McCarty M, Yang XD, et al. Fully humanized neutralizing antibodies to interleukin-8 (ABX-IL8) inhibit angiogenesis, tumor growth, and metastasis of human melanoma. Am J Pathol. 2002; 161:125–34. [PubMed: 12107097]

75. Xu J, Escamilla J, Mok S, David J, Priceman S, West B, et al. CSF1R signaling blockade stanches tumor-infiltrating myeloid cells and improves the efficacy of radiotherapy in prostate cancer. Cancer Res. 2013; 73:2782–94. [PubMed: 23418320]

76. Phase 1 study of IMC-CS4, a monoclonal antibody targeted to the CSF-1 receptor (CSF-1R), in subjects with advanced solid tumors refractory to standard therapy or for which no standard

therapy is available. Eli Lilly. 2011. Internet. Available from: http://clinicaltrials.gov/show/NCT01346358

77. Open-label, multicenter, dose escalation phase Ia/Ib study with expansion phase to evaluate safety, pharmacokinetics and activity of RO5509554, administered as an intravenous infusion as monotherapy and in combination with paclitaxel in patients with advanced solid tumors. Hoffmann-LaRoche. 2011. Internet. Available from: http://clinicaltrials.gov/show/NCT01494688
78. A Phase 1 study to assess safety, pharmacokinetics, and pharmacodynamics of PLX3397 in patients with advanced, incurable, solid tumors in which the target kinases are linked to disease pathophysiology. Plexxikon. 2009. Internet. Available from: http://clinicaltrials.gov/show/NCT01004861
79. A phase 1/2 safety and efficacy study of orally administered PLX3397 in adults with relapsed or refractory acute myeloid Leukemia (AML). Plexxikon. 2011. Internet. Available from: http://clinicaltrials.gov/show/NCT01349049
80. A phase 2 study of orally administered PLX3397 in patients with recurrent glioblastoma. Plexxikon. 2011. Internet. Available from: http://clinicaltrials.gov/show/NCT01349036
81. A phase 1b study to assess the safety of PLX3397 and paclitaxel in patients with advanced solid tumors. Plexxikon. 2012. Internet. Available from: http://clinicaltrials.gov/show/NCT01525602
82. Enhancing efficacy of chemotherapy in triple negative/basal-like breast cancer by targeting macrophages: a multicenter phase Ib/II study of PLX 3397 and eribulin in patients with metastatic breast cancer. Plexxikon. 2012. Internet. Available from: http://clinicaltrials.gov/show/NCT01596751
83. An open label phase 1b/2 study of orally administered PLX3397 in combination with radiation therapy and temozolomide in patients with newly diagnosed glioblastoma. Plexxikon. 2012. Internet. Available from: http://clinicaltrials.gov/show/NCT01790503
84. A phase 1b open label, dose escalation study to assess safety, pharmacokinetics, pharmacodynamics, and antitumor activity of PLX3397 in combination with vemurafenib in V600-mutated BRAF unresectable or metastatic melanoma. Plexxikon. 2013. Internet. Available from: http://clinicaltrials.gov/show/NCT01826448
85. Galluzzi L, Senovilla L, Zitvogel L, Kroemer G. The secret ally: immunostimulation by anticancer drugs. Nat Rev Drug Discov. 2012; 11:215–33. [PubMed: 22301798]
86. Rakhmilevich AL, Buhtoiarov IN, Malkovsky M, Sondel PM. CD40 ligation in vivo can induce T cell independent antitumor effects even against immunogenic tumors. Cancer Immunol Immunother. 2008; 57:1151–60. [PubMed: 18214476]
87. Van De Voort TJ, Felder MA, Yang RK, Sondel PM, Rakhmilevich AL. Intratumoral delivery of low doses of anti-CD40 mAb combined with monophosphoryl lipid a induces local and systemic antitumor effects in immunocompetent and T cell-deficient mice. J Immunother. 2013; 36:29–40. [PubMed: 23211623]
88. Alderson KL, Luangrath M, Elsenheimer MM, Gillies SD, Navid F, Rakhmilevich AL, et al. Enhancement of the anti-melanoma response of Hu14.18K322A by alphaCD40 + CpG. Cancer Immunol Immunother. 2013; 62:665–75. [PubMed: 23151945]
89. Galluzzi L, Vacchelli E, Eggermont A, Fridman WH, Galon J, Sautes-Fridman C, et al. Trial Watch: Experimental Toll-like receptor agonists for cancer therapy. Oncoimmunology. 2012; 1:699–716. [PubMed: 22934262]
90. Link MP, Goorin AM, Miser AW, Green AA, Pratt CB, Belasco JB, et al. The effect of adjuvant chemotherapy on relapse-free survival in patients with osteosarcoma of the extremity. N Engl J Med. 1986; 314:1600–6. [PubMed: 3520317]
91. Kleinerman ES. Biologic therapy for osteosarcoma using liposome-encapsulated muramyl tripeptide. Hematol Oncol Clin North Am. 1995; 9:927–38. [PubMed: 7490249]
92. Meyers PA, Schwartz CL, Krailo MD, Healey JH, Bernstein ML, Betcher D, et al. Osteosarcoma: the addition of muramyl tripeptide to chemotherapy improves overall survival--a report from the Children's Oncology Group. J Clin Oncol. 2008; 26:633–8. [PubMed: 18235123]
93. Buhtoiarov IN, Lum H, Berke G, Paulnock DM, Sondel PM, Rakhmilevich AL. CD40 ligation activates murine macrophages via an IFN-gamma-dependent mechanism resulting in tumor cell destruction in vitro. J Immunol. 2005; 174:6013–22. [PubMed: 15879094]

94. Lum HD, Buhtoiarov IN, Schmidt BE, Berke G, Paulnock DM, Sondel PM, et al. Tumoristatic effects of anti-CD40 mAb-activated macrophages involve nitric oxide and tumour necrosis factor-alpha. Immunology. 2006; 118:261–70. [PubMed: 16771862]

95. Lum HD, Buhtoiarov IN, Schmidt BE, Berke G, Paulnock DM, Sondel PM, et al. In vivo CD40 ligation can induce T-cell-independent antitumor effects that involve macrophages. J Leukoc Biol. 2006; 79:1181–92. [PubMed: 16565324]

96. Buhtoiarov IN, Lum HD, Berke G, Sondel PM, Rakhmilevich AL. Synergistic activation of macrophages via CD40 and TLR9 results in T cell independent antitumor effects. J Immunol. 2006; 176:309–18. [PubMed: 16365423]

97. Buhtoiarov IN, Sondel PM, Wigginton JM, Buhtoiarova TN, Yanke EM, Mahvi DA, et al. Anti-tumour synergy of cytotoxic chemotherapy and anti-CD40 plus CpG-ODN immunotherapy through repolarization of tumour-associated macrophages. Immunology. 2011; 132:226–39. [PubMed: 21039467]

98. Beatty GL, Chiorean EG, Fishman MP, Saboury B, Teitelbaum UR, Sun W, et al. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. Science. 2011; 331:1612–6. [PubMed: 21436454]

99. Woo SR, Corrales L, Gajewski TF. Innate Immune Recognition of Cancer. Annu Rev Immunol. 2015

100. Klohn PC, Wuellner U, Zizlsperger N, Zhou Y, Tavares D, Berger S, et al. IBC's 23rd Annual Antibody Engineering, 10th Annual Antibody Therapeutics international conferences and the 2012 Annual Meeting of The Antibody Society: December 3–6, 2012, San Diego, CA. MAbs. 2013; 5:178–201. [PubMed: 23575266]

101. Albertini MR, Hank JA, Sondel PM. Native and genetically engineered anti-disialoganglioside monoclonal antibody treatment of melanoma. Cancer Chemother Biol Response Modif. 2005; 22:789–97. [PubMed: 16110641]

102. Yamane BH, Hank JA, Albertini MR, Sondel PM. The development of antibody-IL-2 based immunotherapy with hu14.18-IL2 (EMD-273063) in melanoma and neuroblastoma. Expert Opin Investig Drugs. 2009; 18:991–1000.

103. Luedke E, Jaime-Ramirez AC, Bhave N, Carson WE 3rd. Monoclonal antibody therapy of pancreatic cancer with cetuximab: potential for immune modulation. J Immunother. 2012; 35:367–73. [PubMed: 22576341]

104. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. Nat Med. 2000; 6:443–6. [PubMed: 10742152]

105. Lu YC, Yao X, Crystal JS, Li YF, El-Gamil M, Gross C, et al. Efficient identification of mutated cancer antigens recognized by T cells associated with durable tumor regressions. Clin Cancer Res. 2014; 20:3401–10. [PubMed: 24987109]

106. Tran E, Turcotte S, Gros A, Robbins PF, Lu YC, Dudley ME, et al. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. Science. 2014; 344:641–5. [PubMed: 24812403]

107. Marabelle A, Kohrt H, Caux C, Levy R. Intratumoral immunization: a new paradigm for cancer therapy. Clin Cancer Res. 2014; 20:1747–56. [PubMed: 24691639]

108. Kohrt HE, Houot R, Goldstein MJ, Weiskopf K, Alizadeh AA, Brody J, et al. CD137 stimulation enhances the antilymphoma activity of anti-CD20 antibodies. Blood. 2011; 117:2423–32. [PubMed: 21193697]

109. Kohrt HE, Colevas AD, Houot R, Weiskopf K, Goldstein MJ, Lund P, et al. Targeting CD137 enhances the efficacy of cetuximab. J Clin Invest. 2014; 124:2668–82. [PubMed: 24837434]

110. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010; 363:711–23. [PubMed: 20525992]

111. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. Curr Opin Immunol. 2012; 24:207–12. [PubMed: 22236695]

112. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med. 2013; 369:134–44. [PubMed: 23724846]

113. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013; 369:122–33. [PubMed: 23724867]

114. Weiss JM, Subleski JJ, Back T, Chen X, Watkins SK, Yagita H, et al. Regulatory T cells and myeloid-derived suppressor cells in the tumor microenvironment undergo Fas-dependent cell death during IL-2/alphaCD40 therapy. J Immunol. 2014; 192:5821–9. [PubMed: 24808361]

Table 1

Examples of Monoclonal Antibodies (mAbs) with clinical antitumor efficacy, acting via innate immune mechanisms for Cancer Treatment

Name	Target	Indication	Predominant Innate Immune Mechanism of Action
Alemtuzumab	CD52	B-cell CLL, CTCL	CMC
Cetuximab	EGFR	Head/Neck and Colorectal Cancers	ADCC
Dinutuximab	GD2	Neuroblastoma	ADCC
Obinutuzumab	CD20	CLL	ADCC
Ofatumumab	CD20	CLL	ADCC
Panitumumab	EGFR	Colorectal Cancers	ADCC
Pertuzumab	Her2	Breast Cancer	ADCC
Rituximab	CD20	B-cell NHL	ADCC
Trastuzumab	Her2/Neu	Breast Cancer	ADCC

CLL, chronic lymphocytic leukemia; CTCL, cutaneous T-cell lymphoma; CMC, complement mediated cytotoxicity; EGFR, epidermal growth factor receptor; ADCC, antibody dependent cell-mediated cytotoxicity; NHL, non-Hodgkin lymphoma